lung GVHD is currently still on etanercept and stable off steroids. Five patients are still alive currently. Three patients had SD and one patient had PD on etanercept. Causes of death included cGVHD (n=2), disease progression (n=1) and unknown cause (n=1). **Conclusion:** In this preliminary evaluation, etanercept was well tolerated and had activity in patients with cGVHD of the skin, and potentially in some cases with visceral involvement and failing corticosteroids. Further dosing and efficacy studies earlier in the treatment of patients with cGVHD are warranted.

297

ESTABLISHMENT OF A CHIMERIC NOD-Scid/IL2Rγc^{null} TRANSPLANTATION-MODEL TO EVALUATE GRAFT-VS-HOST AND GRAFT-VS-LEUKEMIA IMMUNE RESPONSES OF EX VIVO MODIFIED HUMAN T LYMPHOCYTE GRAFTS

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Donor lymphocyte graft engineering to abrogate graft-vs-host (GVH) reactivity while improving graft-vs-leukemia (GVL) immunity is of particular interest in allogeneic hematopoietic stem cell transplantation. We have recently described a protocol to achieve short-term expansion of donor-derived leukemia-reactive T cells ex vivo followed by CD137-mediated selective depletion of alloreactivity (SAD) to major and ubiquitously expressed minor histocompatibility antigens using allogeneic fibroblasts[1]. For evaluating GVH and GVL immune responses of modified donor T cells in vivo we have now used NOD-scid IL2Rγc^{null} mice to establish a chimeric transplantation model. GVH reactivity after SAD was examined by subcutaneously implanting skin substitutes composed of a collagen-based matrix which contained 6×10^5 human primary fibroblasts derived from the same pool previously used for SAD. Upon implantation, substitutes remained viable, induced angiogenesis and could be dissolved to cell suspensions for further analyses when removed 3 weeks after implantation. Following intravenous injection of 1.5×10^7 preactivated untreated allogeneic human CD3$^+$ T cells per mouse, 7–10% of T cells migrated into the skin substitutes explanted 14 days post adoptive transfer. Such an enrichment of T cells was not observed when the same number of CD137-allodepleted T cells were injected. Since xenogenic GVH reactivity was observed depending on the amount of T cells used current studies investigate whether residual murine antigen-presenting cells initiate xeno-reactivity and address its impact on the migration of T cells into the skin substitutes. To study GVL immunity we further transplanted different amounts of acute myeloid leukemia (AML) cells derived from primary AML patients (M4 and M5 subtype) into NOD-scid IL2Rγc^{null} mice. Eight weeks after injection 61% and 17% of isolated spleen and bone marrow cells, respectively, stained positive for a shared HLA-class-I epitope and also expressed CD34 as measured by flow cytometry. Currently, studies are in progress to examine immune responses to AML by adoptive transfer of human AML-stimulated allodepleted T cell lines devoid of alloreactivity to AML HLA-matched fibroblasts. In summary, our NOD-scid/IL2Rγc^{null} transplantation model may be a valuable tool for evaluating GVH and GVL immunity in vivo following modifications of donor T lymphocytes grafts in vitro.

[1] Wehler T and Nonn M, et al. 2006. Blood;epub. Aug. 24.

298

ALLOANTIGENS EXPRESSION ON HOST NON-HEMATOPOIETIC CELLS LEADS TO DONOR T CELL EXHAUSTION AND REDUCES GVL EFFECTS

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We previously showed that alloantigen expression on host non-hematopoietic cells impaired graft-versus-leukemia (GVL) effects after allogeneic hematopoietic stem cell transplantation using chimeric mice expressing alloantigens on hematopoietic cells alone. C3.Sw (H-2^b) mice were lethally irradiated and injected with T cell depleted bone marrow cells (TCD-BM) from multiple minor histocompatibilitiy antigens (mHAs) disparate B6 (H-2^b) donors. These chimeric mice (BC chimeras), expressing B6-derived mHAs only on hematopoietic cells, were lethally irradiated and transplanted with TCD-BM and CD8$^+$ T cells from C3.Sw donors together with B6-derived leukemia cells, EL-4. In controls we used B6→B6 chimeras (BB chimeras), expressing B6-derived mHAs on both hematopoietic and non-hematopoietec cells, as recipients. BB chimeras transplanted with TCD-BM + CD8$^+$ T cells from C3.Sw exhibited a significant GVL effect but died from leukemia significantly earlier than BC chimeras. To further confirm that alloantigen expression on non-hematopoietic cells impairs GVL activity, similar experiments were performed using B6→B6-β2m deficient chimeras (B- chimeras), lacking functional MHC class I molecules on non-hematopoietic cells. Leukemia mortality was significantly reduced in B- chimeras compared to BB chimeras, thus confirming that alloantigen expression on host non-hematopoietic cells impairs GVL effects. To elucidate the mechanism of the reduced GVL effects in BB chimeras, T cells were isolated from lymph nodes and spleen of chimeras after BMT. Numbers of donor CD8$^+$ T cells in lymph nodes and spleens of BB recipients decreased much earlier than in BC chimeras in association with an enhanced apoptosis. CTL activities against EL-4 significantly reduced in BB recipients compared to BC and B- chimeras. PD-1 expressions on CD8$^+$ T cells from BB chimeras were significantly enhanced compared to those from BC chimeras. These results suggest that alloantigen expression on host non-hematopoietic cells leads to exhaustion and dysfunction of donor T cells and impairment of GVL effects.

299

THE ROLE OF SNPS WITHIN RECEPTORS OF INNATE IMMUNITY IN OUTCOME FOLLOWING ALLOGENEIC STEM CELL TRANSPLANTATION: SYNERGISM BETWEEN *TLR5-STOP* AND *NOD2/CARD15*?

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Our group recently reported an association of single nucleotide polymorphisms (SNPs) within *NOD2/CARD15*, an important intracytoplasmatic receptor of the bacterial ligand muramyl-dipeptide, with severe GvHD, treatment related mortality (TRM) and outcome following allogeneic stem cell transplantation (SCT). As these studies suggested a major role of dysregulated epithelial inflammation, we now tested the role of SNPs within further receptors involved in cellular sensing of bacterial ligands. SNPs reported for *TLR2*, *TLR3*, *TLR4*, *TLR5* and *TLR9* were assessed in recipient (R) and donor (D) DNA from 259 HLA-identical sibling and 294 matched unrelated donor transplants. Acute GvHD grade III/IV, 1-yr- and overall TRM as well as overall survival were correlated with results of SNP typing, and the influence of major clinical risk factors as well as previously assessed presence or absence of *NOD2/CARD15* SNPs was investigated with univariate and multivariate analyses.

Whereas major SNPs within *TLR2*, *3*, *4* and *9* failed to show clear associations, the presence of a *TLR5-stop* codon in the recipient (n=58) showed a trend for higher severe GvHD (22%